PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P044976PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL 03/00170	07.03.2003	07.03.2002
International Patent Classification (IPC) or both national classification and IPC		
C12N5/06		
Applicant		
ACADEMISCH ZIEKENHUIS BIJ DE UNIVERSITEIT ..et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24.09.2003	16.06.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Pilat, D Telephone No. +49 89 2399-8668

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00170

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-49 as originally filed

Claims, Numbers

1-19 received on 06.02.2004 with letter of 06.02.2004

Drawings, Sheets

1/12-12/12 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☐ claims Nos.

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-19 partially

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-5,7-19
Inventive step (IS)	Yes:	Claims	
	No:	Claims	6
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations

   see separate sheet

The examination is being carried out on the **following application documents:**

Text for the Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PL PT RO SE SI SK TR

Description, pages:

1-49 as originally filed

Claims, No.:

1-19 as received on 06/02/2004 with letter of 06/02/2004

Drawings, sheets:

1/12-12/12 as originally filed

Ad Section I: Basis of the report

Reference is made to the following documents:

D1: US 2001/033836 A1 (SYMONDS GEOFF ET AL) 25 October 2001 (2001-10-25)

D2: SETOGUCHI K ET AL: 'Antigen-specific T cells transduced with IL-10 ameliorate experimentally induced arthritis without impairing the systemic immune response to the antigen' JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 165, no. 10, 15 November 2000 (2000-11-15), pages 5980-5986, XP002196421 ISSN: 0022-1767

D3: MORITANI MAKI ET AL: 'Prevention of adoptively transferred diabetes in nonobese diabetic mice with IL-10-transduced islet-specific Th1 lymphocytes: A gene therapy model for autoimmune diabetes' JOURNAL OF CLINICAL INVESTIGATION, NEW YORK, NY, US, vol. 98, no. 8, 1996, pages 1851-1859, XP002152055 ISSN: 0021-9738

D4: MATHISEN PETER M ET AL: 'Treatment of experimental autoimmune encephalomyelitis with genetically modified memory T cells.' JOURNAL OF

EXPERIMENTAL MEDICINE, vol. 186, no. 1, 1997, pages 159-164, XP002241533 ISSN: 0022-1007.

1. Amendments (Article 34 (2) (b) PCT)

1. The amendments submitted with the applicant's letters on the 6.2.2004, appear to be admissible.

Ad Section II :Priority

2) The priority document pertaining to the present application was not available at the time of establishing this written opinion. Hence, it is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the P document cited in the international search report could become relevant to assess whether all claims satisfy the criteria set forth in Article 33(1) PCT.

Ad Section III :Non-establishment of opinion

Insofar as the applicant did not pay additional search fees, the international search has been carried out on the first invention identified which refers back to a method for producing mononuclear cells overexpressing IL-10, wherein the composition comprising peripheral blood mononuclear cells is enriched in lymphocytes, i.e. on claims 1-19 partially (see also Form 210). As a consequence, **the international preliminary examining report is restricted to the invention that has been searched** (see also section III).

Ad Section V :Reasoned statement under Rule 66.2(a)(ii); citations and explanations supporting such statement

2. Novelty (Article 33 (2) PCT)

2.1 D1 discloses, in paragraph 59, the method of present claims 1-3 comprising the steps of collecting peripheral blood, and the CD4+ cells isolated. CD4+ cells reactive to collagen or other antigens within synovial fluid are expanded in the presence of IL-2. The gene encoding an anti-inflammatory cytokine molecule e.g.

IL-10 is then introduced into the CD4+ cells via a retroviral vector. The cells are then expanded and recovered before being injected into patients (see example 1 §59).
It is emphasized that the method as described in D1 isolates mononuclear cells, i.e CD4+ cells, overexpressing the polypeptide having IL-10 activity. They are not specific for a predetermined antigen. The isolated mononuclear cells in D1 are reactive to multiple different antigens, for example collagen type II or antigens within the synovial fluid and thus are not specific for a predetermined antigen.

Thus, in view of the content of D1, claims 1-3 are not new.

This conclusion is not reversed by the expression "unique epitopic locus", which means the surface area on a single antigenic molecule, or formed by a plurality of antigenic molecules, which (a) exists at or near the location of the disorder in the afflicted subject (b) does not exist at or near this site in an unafflicted subject and (c) can be recognized by and specifically bound to CD4+ cells present in the afllicted subject.

2.2 Dependent claims 4-9 do not appear to be new either. It seems that the mononuclear cells must have once proliferated at one stage prior to step (b) and that this proliferation occurred necessarily in the presence of at least one proliferating agent (see D1 §21 steps a) to d)). It seems equally that, at any time, subsequent to (b) mononuclear cells needed to be enriched in order to be used in therapy (see D1 example 1, §59).

2.3 The compositions obtained by the previous method as described in D1, comprise mononuclear cells which are stated to be included in pharmaceutical compositions (see e.g. D1 p.2 §22). This content anticipates claims 10-14.

2.4 D1 discloses the treatment of disorders, using the composition obtained by the method of D1, like rheumatoid arthritis, IDDM, MS Crohn's disease, psoriasis, Grave's disease, Hashimoto thyroiditis and autoimmune uveitis (see D1 p.3 §28-32 and §34). D1 discloses also that interleukin 10, is the therapeutic gene for treating rheumatoid arthritis and diabetes (see p.3 §37). This teaching anticipates claims 15-19.

3. Inventive step (Article 33 (3) PCT)

3.1 The mere selection of a proliferating agent among CD3/CD28 or PHA does not involve an inventive step, but amounts to an obvious choice the skilled person would have performed in order to activate proliferation of mononuclear cells. Thus, claim 6 is trivial.

Ad Section VIII : Certain observations on the international application.

4. Clarity (Article 6 PCT)

4.1 Claim 13 should be characterized by its essential features. Claim 13 does not exclude prior art T cells. Indeed, it cannot be excluded that prior art compositions comprising T cells containing an IL-10 transgene are equally capable to decrease proliferation of autologous responder cells and/or decrease production of the pro-inflammatory cytokine IL-12 by dendritic cells.

4.2 Claim 19 refers to a disease which is ambiguously characterized in that it is associated, to what extent is unclear, to a undesired activation and/or expansion of T cells. The last member of the sentence is equally open to interpretation insofar as the skilled person cannot directly and unambiguously derive which diseases fall under this expression "undesired activation or expansion".

4.3 Claim 1 refers to a method for producing mononuclear cells overexpressing IL-10 comprising the step of ... (b) introducing an expression construct comprising a nucleotide sequence encoding a polypeptide having IL-10 activity, ... It is emphasized that it is not yet defined which IL-10 activity is to be considered. Due to the wording of claim 1 any polypeptide encoding at least one IL-10 activity falls under the scope of protection. It remains questionable how a polypeptide having only one same activity with IL-10 would be capable to produce mononuclear cells overexpressing IL-10 as claimed in claim 1.

Claims

1. A method for producing mononuclear cells overexpressing IL-10, wherein the method comprises the steps of:

(a) providing a composition comprising peripheral blood mononuclear cells (from a mammal);

(b) introducing an expression construct comprising a nucleotide sequence encoding a polypeptide having IL-10 activity into at least part of the mononuclear cells; and,

(c) recovery of mononuclear cells overexpressing the polypeptide having IL-10 activity.

2. A method according to claim 1, wherein composition comprising peripheral blood mononuclear cells is enriched for a subfraction of the peripheral blood mononuclear cells.

3. A method according to claim 2, wherein the subfraction of mononuclear cells is selected from the group consisting of lymphocytes, B cells, T cells, $CD4^+$ cells, macrophages, monocytes or dendritic cells (DC).

4. A method according to any one of claims 1 - 3, wherein prior to step (b) the mononuclear cells are proliferated.

5. A method according to claim 4, wherein the mononuclear cells are proliferated in the presence of a proliferating agent.

6. A method according to claim 5, wherein the proliferating agent is at least one of CD3/CD28 or PHA.

7. A method according any one of claim 1 - 6, wherein subsequent to (b) the mononuclear cells are enriched for a subfraction of mononuclear cells.

8. A method according to claim 7, wherein the subfraction of mononuclear cells is selected from the group consisting of lymphocytes, B cells, T cells, $CD4^+$ cells, macrophages, monocytes or dendritic cells (DC).

9. A method according to any one of claims 1 - 8, wherein subsequent to step (b) the mononuclear cells are enriched for cells (over)expressing the IL-10 transgene.

10. A method for producing a pharmaceutical composition comprising mononuclear cells overexpressing IL-10, mixing cells obtained in above claims with suitable pharmaceutical carrier.

11. A composition comprising mononuclear cells containing an IL-10 transgene.

12. A composition according to claim 11, whereby the composition comprises T cells containing an IL-10 transgene.

13. A composition according to claim 12, whereby the T cells phenotypically mimic regulatory T cells in that the T cells decrease proliferation of autologous responder cells and/or decrease production of the pro-inflammatory cytokine IL-12 by dendritic cells.

14. A composition according to any one of claims 11 - 13, wherein the composition is a pharmaceutical composition comprising in addition to the mononuclear cells a pharmaceutically acceptable carrier.

15. A method of treating a disease associated with undesired activation and/or expansion of T cells, wherein the method comprises administering a pharmaceutical composition according to claim 14 to a subject suffering from a disease associated with undesired activation and/or expansion of T cells.

16. A method according to claim 15, wherein the disease associated with undesired activation and/or expansion of T cells is a Th1-mediated disease, more preferably Th1-mediated inflammatory diseases.

17. A method according to claim 16, wherein the Th1-mediated disease is selected from the group consisting of Crohn's disease, reactive arthritis, insulin-dependent diabetes, colitis, pancreatitis, an lung, an inflammatory eye disease, multiple sclerosis,

Hashimoto's thyroiditis, Grave's disease, chronic articular reumatism, contact dermatitis, psoriasis, graft rejection, graft versus host disease, and sarcoidosis.

18. A method according to any one of claims 15 - 17, wherein a the composition comprising the mononuclear cells is administered in a therapeutically effective amount.

19. Use of IL-10 overexpressing mononuclear cells as obtained by any of the methods of claims 1 - 9, in the manufacture of a medicament for use in the treatment of disease associated with undesired activation and/or expansion of T cells.